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PHYTOPATHOLOGY

VOLUME XIII NUMBER 7

JULY, 1923.

THE BIOLOGICAL BASIS OF FOREIGN PLANT QUARANTINES

W. A. Orton and R. Kent Beattie[1]

Plant diseases and insect pests are often limiting factors in crop production and their prevention or control is one of the major problems of agriculture.

In all developed countries the introduction and spread of such pests in the past has caused such heavy losses, even to the point of restriction or abandonment of important crop industries, and now imposes such a heavy annual tax for sprays, seed treatments, cultural control and the substitution of resistant varieties of cultivated plants that the people have invoked governmental aid to apply official regulatory measures which will protect them from additional burdens of this character in the future.

The efforts that are being made along this line may be grouped under four heads, according to the prevalence status of the pests involved:

1. *State Inspection and Certification of Plants and Plant Products.* All progressive countries maintain inspection services for the general control of insects and plant diseases, including those already common, widespread or endemic, through supervision of commerce in nursery stock, seeds and plant products.

2. *Eradication.* To wipe out newly introduced parasites presenting an unusual menace to our crop industries. Two outstanding illustrations are the fight waged against citrus canker in Florida and that against the pink bollworm of cotton in Louisiana and Texas. The eradication method has also been applied successfully to outlying "long jump" infestations of gypsy moth and other insects. As a rule, it is extraordinarily difficult to eradicate an insect or disease that has gained a firm foothold in a region favorable for its maintenance.

3. *Domestic Quarantines.* Restriction of new introductions by local or domestic quarantines drawn around an area where a new pest from a foreign country has become established. Examples are the quarantine against the gypsy and browntail moth in New England, the Japanese beetle in New Jersey and Pennsylvania, and potato wart in three states.

[1] Read at the joint meeting of the American Phytopathological Society and American Association of Economic Entomologists, Boston, Mass., December 30, 1922.

In the same category may be placed the efforts made by various states to keep out pests established in other, often distant states. Arizona regulates against cotton pests from the East, alfalfa weevil from the North, citrus pests from the South, and grape phylloxera from the West. In such cases a barrier has been erected around the pest-free area rather than around the infested area.

These local or domestic quarantines are temporary expedients, whereby the spread of a pest is slowed up or held back at heavy cost and much inconvenience, but domestic dispersal is almost unrestrainable and, sooner or later, the point is reached where the newly established pest acquires the status of other widely distributed parasites, to which we have to adjust ourselves forever.

4. *Foreign Quarantines.* These seek to exclude the plant pests of other countries on the principle that frontier boundaries are the most effective fighting lines. It is primarily with this problem that we are to deal in this paper.

We stand in danger of subjecting ourselves to ineffective and hampering regulations without adequate gain unless the quarantine procedure is based upon a firm scientific foundation of sound biological principles derived from properly coördinated facts relating to the nature of the parasites to be dealt with, their country of origin and geographic distribution, their host relations, and the native home of the host, prevalence, climatic relations, manner of spread, and other factors. More studies of this character need to be made, but enough is known to enable some general principles to be established.

FOREIGN QUARANTINE PROBLEMS DISTINCT

It is our purpose to emphasize that the application of biological principles to plant quarantines must lead to a primary distinction between the problem of control of spread within a given area, which has been termed "continuous spread," and the long distance transfer that has been termed "discontinuous spread" by Butler[1] in his excellent monograph on this subject.

It was pointed out in 1914[2] that we have to deal with an *intercontinental* problem and that to safeguard the exchange of plants and their products between countries situated in different continents more stringent precautions might become necessary.

[1] Butler, E. J. The dissemination of parasitic fungi and International Legislation. Memoirs of the Department of Agriculture in India, **9**: 1–73, 1917.

[2] Orton, W. A. The biological basis of international phytopathology. Phytopathology, **3**: 327, 333, Feb. 1914.

That in establishing plant quarantine policies the problems of restricting parasites from foreign lands must be grouped separately from the problems of preventing or delaying dispersal of pests already locally established may appear self-evident to some, but that this important point is not yet properly acknowledged is shown by the report of the last International Phytopathological Convention held in Rome in 1914, which makes no distinction between contiguous and non-contiguous countries, with respect to the methods to be adopted to prevent the spread of plant parasites. The representations made by European delegations to the Federal Horticultural Board during the past year have likewise ignored this basic principle, and have included proposals for intercontinental exchange of plants that we must regard as extremely unscientific and dangerous.

THE BASAL PRINCIPLE OF BIOLOGICAL EQUILIBRIUM

The plant parasites of one continent or natural plant zone are likely to be more virulent and destructive when introduced into another continent or plant zone than they are at home, where they are in equilibrium with their hosts and associates. This principle of biological equilibrium in Nature is best understood by considering from the viewpoint of evolution the results of the complex interactions of organisms, the struggle for existence, the influence of animals on plants, of plants on animals, and the disturbing effect of man, who seeks to exterminate some animals and plants and increases the number of other organic elements in the equation, even bringing in foreign organisms to participate in the mixup.

In the course of evolution many marvelous adaptations to environment arise and many complex interrelations develop. Organisms are sheltered by some of their associates and preyed upon by others. They become dependent upon certain kinds of food, and increase or diminish in numbers with the abundance of this food.

The type of relations that concerns us is *parasitism*, wherein one organism lives upon and at the expense of another. A classification of parasites on the basis of their adaptation to their hosts reveals the course of evolution, which has progressed from saprophytism, through wound parasites and weakling parasites, toward a stage of closer adaptation between host and parasite till symbiosis is reached, a relation where the parasite does little or no injury or even stimulates the host to larger growth till the reproduction and dissemination of the parasite is assured.

There is a tendency for the fungus or bacterium to reduce its toxin formation, and on the part of the host to develop a degree of immunity, so that a balance is established between the two, with a lessened injury

to the host and a more highly perfected mechanism of dispersal for the parasite. With insects, the balance is established more often through the influence of parasites, predatory insects, and birds.

This equilibrium is dynamic, not static, ever changing, but tending to preserve the status quo, and in the establishment of equilibrium the time element is important. While the restoration is slowly taking place in a new country much economic damage often occurs.

So it comes that the natural parasites of a region are not highly destructive to the plants of that region, as a general rule, but when one of these parasites is transferred to a different plant zone, it may come into contact with a new set of organisms and find new hosts that are not resistant.

In considering the problems that arise when a new plant disease or insect is discovered to have been introduced, or in estimating the risk presented by a foreign parasite, it is well to begin by ascertaining:

1. The country where the parasite is endemic.
2. Its native or original host.
3. The geographic distribution of this host.
4. Its relationship to other economic plants in our country.
5. If the host is cultivated here, to what extent and for how long it has been subjected to plant breeding that may have resulted in the production of distinctively American strains whose resistance to the parasite is unknown.

Various types of disturbance of biological equilibrium by the transfer of parasites from one plant zone to another occur amd may be illustrated by notable examples.

I. A parasite introduced from one continental zone to another finds susceptible hosts. A conspicuous instance where a foreign fungus has found an American host more susceptible than its original host is the chestnut bark disease, *Endothia parasitica*, which occurs in Asia on *Castanea mollissima* as a relatively minor trouble, but introduced into the United States by accident, has attacked our *Castanea dentata* most virulently and will practically destroy all the chestnut forests of the country before it stops. In America this Asiatic parasite has found the European chestnut, *Castanea vesca*, as easy prey as the American and if introduced into Europe it may yet repeat there the devastation we have witnessed here. Europe has already suffered heavily from introductions of this type. The wine grape, *Vitis vinifera*, has been scourged in succession by the phylloxera, the downy mildew, *Plasmopara viticola*, and the black rot, *Guignardia bidwellii*, to all of which American species of Vitis are resistant.

European gooseberries are found to be susceptible to the American mildew, which is becoming widespread in Europe. We have long known that the European varieties were relative failures in the United States on account of this mildew, to which American species are resistant.

The white pine blister rust, *Cronartium ribicola*, a native of Europe on *Pinus cembra* and other species is very destructive to *Pinus strobus* in the United States, and another American species, *Pinus monticola*, is several times as susceptible as *P. strobus*. This had been forecasted prior to the actual introduction from the injury done to American white pines when planted in Europe.

The hollyhock, a native of China, met the rust, a South American fungus, in Europe and its culture was nearly stopped. The disease later spread to North America and repeated its destruction here.

In this general category belong also numerous cases of insect transfers to other zones where, relieved of the enemies that have held them in check at home, the invaders have multiplied with great rapidity. The cottony cushion scale, *Icerya purchasi*, for example, came to California from the Orient and was not controlled till its enemy, the Australian lady bird *Vedalia cardinalis*, was brought over and a new biological equilibrium established. Similarly the Japanese beetle, *Popilia japonica*, the European corn borer, *Pyraustra nubialis*, the gipsy moth, *Porthetria dispar*, the brown tail moth, *Euproctis chrysorrhoea*, and the alfalfa weevil, *Hypera postica* came to this country without their natural enemies. In combating them efforts have frequently been made to discover and to introduce artificially their enemies.

II. A plant, carried from one continental zone to another, is attacked by new parasites. The culture of maize in Java and the Philippines is well nigh impossible because of the corn mildews, *Sclerospora spontanea*, *S. maydis*, *S. sacchari*, and *S. Philippinensis*, which are oriental species, the latter being native on *Saccharum spontaneum* and *Miscanthus japonicus*.

When the common potato in its spread from South America reached the Rocky Mountains of the north, it was attacked by the Colorado beetle, *Doryphora decemlineata*, which found the newcomer more palatable than the Solanums on which it had been accustomed to feed.

Of the same type is the situation we are now facing in Arizona, where a native weevil, *Anthonomus grandis* var. *Thurberiae*, related to the cotton boll weevil, has already shown an inclination to move over to the cultivated cotton, a relative of its wild host, *Thurberia thespesoides*. Since the Thurberia weevil is acclimated to the dry southwestern climate as the Mexican weevil is not, it would extend the zone of weevil injury as far as cotton is grown.

As already mentioned, the European gooseberries brought to American were attacked by the American mildew and the attempts to establish the culture of European grapes in eastern North America were always failures on account of phylloxera, black rot, and mildew.

III. A cultivated plant, transferred from one continent to another without its parasites, is later followed by them. In the interval, new varieties have arisen which do not possess the high resistance the species originally had. Our American agriculture is based so largely on introduced crops that we have had numerous cases of this type in the past and there is ground for grave apprehension that still others may be brought in. The asparagus rust affords a significant illustration of this phase of this biological principle. Asparagus and its rust are both native Europeans and the disease is not highly injurious on that continent, but the crop came to the United States without the disease and from its earliest culture until about 1896 was free from rust. During this period American varieties were bred and formed the basis of our industry when the rust finally invaded the United States. It soon transpired that the Americans strains of asparagus which had been developed in the absence of the rust and never before had been in contact with the disease were very susceptible, and they were soon wiped out. European strains were imported and resistant types selected from them. These have now replaced the old susceptibles kinds, and an interesting thing has happened. Asparagus rust has dwindled and in places almost disappeared; in other words, we have restored the natural equilibrium such as exists in Europe.

IV. Domestic parasites are sometimes limited in their natural geographic distribution by climatic factors. The black rot of grape, which requires high temperatures for infection, is most at home in the central eastern states. A New England grape from *Vitis labrusca*, the Concord, is more susceptible in that region than are varieties derived from *Vitis rotundifolia*. Pecan scab, *Fusicladium effusum*, is most destructive to varieties from the southwest, Texas for instance, planted in the more humid region of the Atlantic Seaboard.

The vast areas planted to one crop in the United States make for a disturbed equilibrium and afford favorable conditions for the rapid spread of parasites.

The transfer of plant parasites from continent to continent was extremely slow in the early days when the means of communication were scanty and the voyages long, but notable instances have multiplied in proportion as the volume of traffic has increased. World trade is certain to be in a still larger scale in the future, with proportional risk of spreading pests unless scientific control measures are rigidly enforced.

NATURAL BARRIERS AGAINST PLANT PESTS

What are the plant zones within which organic life is in a state of equilibrium? Clearly they are in the main *continental*. The extent to which mountains and deserts restrict the spread of parasites has never been adequately studied but such cases are known. Here is a field to be highly commended to investigators.

It will be clear that the plant relations of two contiguous countries without a natural barrier are different from those of two countries separated by an ocean barrier. In the first instance the endemic parasites are the same and respect no political boundaries. The plants and parasites of the United States have reached their ultimate distribution except as new introductions are still spreading. For the countries of Europe the Rome Convention may be useful. A similar plan of certification is in effect between our states but it will not cover the relation between North America and Europe, and still less between North America and Asia.

Since plant parasites are in dynamic equilibrium with their natural hosts, with a tendency toward reduced injury, it follows that it is now impossible and that it never will be possible to foretell from the behavior of the disease or insect in its native home what it may do when transferred to another continent. Experience has shown that in many cases we may expect it to go over to a near related host and attack this plant with great virulence.

It is important to study in Europe and Asia the diseases of American plants that have been introduced there. For example, we knew in advance that the European blister rust was especially destructive to American white pine, because that tree had been much planted in Europe and had failed there because of the rust.

The effect of the canker due to *Phomopsis pseudotsugae* recently reported from Scotland[1] upon the American species of Pseudotsuga, Larix, Tsuga, and Abies, now growing there deserves careful study in the light of the importance of our western Douglas fir forests.

Most of our cultivated plants are native to other continents, but crop plants are subject to rapid change by breeding and the resistant character previously attained abroad may be dropped or diminished unawares. Since our present day varieties are of American origin, bred for adaptation to our climate and soil, it is to be expected that as additional parasites accidentally become established they will be more destructive to our American bred varieties than they are to the parent

[1] Wilson, Malcolm. A new species of Phomopsis parasitic on the Douglas fir. Trans. Bot. Soc. Edinburgh, 28: 48–50. 1920.

species in the original habitat. The need for strict exclusion is therefore so much the greater.

CROP INTRODUCTION

To promote the agriculture and horticulture of a new country, it is desirable to introduce the crop plants of other lands. It is possible, and highly desirable, to accomplish this without bringing along their parasites. For example, we have the Australian eucalyptus on the Pacific Coast with very few pests, whereas in its native country it has a good many parasites. This is probably due to the fact that our introductions have been by seed rather than by plants. Unfortunately, this freedom from pests can be said of few of our great staple crops. Many diseases of wheat, potatoes, apples, peaches, etc. trace back to the original habitat of these crops. What losses beyond the possibility of estimation might have been saved if national barriers against plant pests had been erected at the start. However, by no means all of the crop pests of other lands have been imported, and we have still many new plants to bring in. It is to be hoped their enemies may be left behind.

At this point we may expect to hear from the man who proposes that it would be better to let all the foreign parasites in and strike a balance with them, developing control method once for all.

Unthinkable! Count the cost of a few more scourges like the chestnut bark disease. Could our corn growers combat the Philippine downy mildew? In his description of the ravages of this disease in the Philippines, Weston describes fields where practically every plant was destroyed and he believes that climatic conditions in our corn belt would favor the mildew. It is difficult enough to establish the culture of a new crop in the absence of its serious pests. It would become impossible if all the enemies were brought in.

Moreover, a biological equilibrium would never get established, for the succession of possible pests from all the ends of the earth is endless. There would always be more to come. We would not learn to cope with one enemy before another would be upon us.

Control of pests is not an attempt to overcome nature's laws. The movement of cultivated plants into new regions is a reassembling of environmental forces (geological, climatic, biological, anthropological) to permit production of material useful to man. The economic plant product is the resultant of these forces. The importation of plants without the importation of pests is merely a reassembling of these forces in a different and just as natural a manner as the importation without regard to the transfer of pests.

WHAT ARE THE FACTORS GOVERNING THE SPREAD OF PLANT PARASITES?

Theorists might stress wind carriage. Volcanic dust has been borne thousands of miles through the air. Why not fungus spores?

But no case of long distance transfer of plant disease by wind has been demonstrated, and the facts of geographic distribution show that diseases frequently have failed to spread even short distances until carried by man. There is a place in the District of Columbia where for twenty years apples and red cedars have been growing in close proximity without a trace of cedar rust, though ten miles away this fungus is a scourge to both hosts. Butler, after an extensive review of the evidence, concludes, that "infection by spores carried through the air from remote centers is not a contingency which needs to be seriously taken into account."

Ballast brought to our shores in the holds of vessels from foreign ports is a bugaboo to the theorists, but no case of disease importation has been traced definitely to it. The practice should be regulated and ballast of live or agricultural soil prohibited. Earth balls on the roots of imported plants stand in a very different relation and are much more dangerous, as they are the resort of many insects and may contain harmful fungi or nematodes.

Spores have been found on birds, and birds fly long distances. Clearly they might spread infection, but the facts of distribution already referred to and studied, of the spread of great plant epidemics, indicate that spread is mainly by continuous dispersal, or in long jumps through human agencies.

PLANT DISEASES SPREAD BY MAN

The *most frequent agent in plant pest dissemination is man*, and the outstanding and altogether most important medium of *transfer is living plants or parts of plants for propagation*. Trees, roots, bulbs, and seeds, taken in a living condition from their place of origin, with parasites attached, are carried to a new location, set out, and brought into growth. The careful moist packing and cultural precautions taken to preserve their vitality likewise insure the perpetuation of the parasite.

Insufficient consideration has been given to dispersal on seed through external or internal infection, which has carried many plant parasites over the world. War needs brought a shipload of castor beans from India to Florida, and with it a Botrytis blight that made the culture of this crop impossible. Investigation proved that the parasite was seed-borne.

Practically all of the pest transfers of which we have accurate record have taken place in this way. In the nursery a large number of related

species of plants are brought together in such a way as to favor continuous spread. If these plants become infected the discontinuous spread of the disease may occur when the nursery stock is shipped to distant parts of the world. Thus in the majority of cases the nursery is the principal center of distribution.

We must lay it down as an unchangeable law of nature that *intercontinental trade* in *plant propagating material* is fundamentally dangerous, and to be held within the narrowest limits compatible with economic need.

In human diseases the same principles apply. The living carrier is a greater risk than air-borne infection. The practices of the most progressive public health authorities show an increasing recognition of this principle.

INSPECTION INADEQUATE

Experience has shown that by an inspection of plants in nurseries, or better in the packing house, a worth while safe-guard is provided for shipments to contiguous states in the same biological zones, since the number of parasites is greatly lessened, but the most rigid inspection can not detect all the insects, as shown by the frequent findings of brown-tail moth nests in certified nursery stock upon its arrival in America from North Europe. How much less likely are the inspectors to discover the presence of fungous and bacterial diseases, due to organisms of minute size and often embedded in the tissue of the host plant, as in the case of white pine blister rust, where an internal infection of seedlings shipped to America from Europe has been detected two or three years later.

There are three biological factors which operate to make inspection inadequate for intercontinental shipments.

1. Human powers of observation are not keen enough to detect 100 per cent of visible infection.
2. Some parasites are internal, without outward manifestation.
3. Organisms common and of minor consequence in one biological zone may become destructive parasites in another biological zone.

The tendency of inspection officials has been to overlook "common species of long standing dispersion," to quote the language of the Rome Convention, which in addition omits from consideration "enemies of plants whose ordinary supports do not exist in the importing countries," and those which do not present:

1. An epidemic character.
2. A destructive or at least very harmful action on cultivated plants.
3. An easy propagation by living plants or parts of living plants.

These considerations are logical only when applied to contiguous countries. To other continents the only effective protection is the nearest approach to absolute exclusion of living plants that economic needs permit. Importations for propagation of new or rare varieties should be under governmental supervision, and if the origin is in unsurveyed countries they should be grown for a period in a quarantine or detention station, and in any case should be given the most effective treatment possible.

TREATMENT METHODS INADEQUATE

Fumigation of plants is a safeguard, but not a complete protection. It has not reached scale insects in tightly wrapped leaves or buds, as in the date palm, nor egg stages of the cane leafhopper, nor insects in moist earth or in packing material around plants. Fumigation has also failed completely to kill the larvae of the twig borer, *Laspeyresia molesta*, in peach twigs and those of the corn borer, *Pyrausta nubilalis*, hidden in the stalks of bales of broom corn.

No method has been devised to disinfect seeds or plants that can be depended upon to reach all fungi and bacteria present, though such treatments are of value in particular cases.

WHAT THEN OF PLANT PRODUCTS FOR FOOD OR MANUFACTURE?

This is an important question, because such commoditites form a large part of our trade, both export, import, and domestic, and extensive restriction might prove more harmful than helpful.

Here again, proof is lacking, except in a few instances, that plant pests are actually disseminated, and these cases are mostly those where the commercial product is a seed, tuber, or part capable of propagation. Cotton seeds are dangerous carriers of pink bollworm. Potatoes introduce disease to the extent that table stock is used for planting. Tuber parasites are transported with particular ease. Apple fruit has not spread San Jose scale. This scale has not become established in northern Europe, although often present on fruit imported from Australia. Pear fruit does not expose the importing country to a risk of pear blight. Fruit flies, however, are carried in commercial fruit and may escape therefrom and are therefore subject to discontinuous spread. Each case is a problem for research and separate consideration.

It highly behooves those interested in building up an intercontinental trade in plant products to take measures to provide a safe foundation for such enterprises, to insure that the importation of a fruit or vegetable or other plant product will not later become a means of exposing North American crops to injury from a new disease or insect.

This foresight can be exercised through scientific research in the country of production, in tropical America, for example, to keep out pests or to keep them down, or to develop methods of culture and packing that will make it impossible for pests to be spread.

SUMMARY

Plant quarantine policies and methods must be founded on nature's laws governing the dispersal of parasites.

The problems of discontinuous spread between countries separated by ocean or other natural barriers are therefore different from those of continuous spread in adjacent countries.

The pests of other continents are fundamentally the most dangerous. Seldom can they be known in advance or completely eliminated by inspection. Consequently intercontinental trade in living plants will always be attended by such grave risks that it should be held within the narrowest limits compatible with economic needs.

FEDERAL HORTICULTURAL BOARD,
WASHINGTON, D. C.

BACTERIAL SPOT OF TOMATO AND PEPPER[1]

MAX W. GARDNER AND JAMES B. KENDRICK[2]

WITH PLATE XIV AND ONE FIGURE IN THE TEXT

At the time of our previous reports (3, 4) on bacterial spot of tomato, the identity of the causal organism of pepper bacterial spot was only partially established and the final report of Miss Doidge on tomato canker in South Africa was not accessible. It is our present purpose to clear up the uncertainty relative to the identity and taxonomy of the causal organism and to record the results of our further study of the disease in Indiana.

HISTORY OF THE DISEASE

The earlier literature was summarized in our previous report (3) in which we described the tomato organism, recorded successful reciprocal cross inoculations with the causal bacteria of the tomato and pepper diseases, and indicated that the organisms were probably identical. A few months before our account was published, Miss Doidge at Pretoria, Transvaal, published a preliminary (1) and a detailed (2) description of a bacterial tomato canker occurring in that region which has proved to be identical with the disease studied in Indiana.

The bacterial spot of pepper described by Sherbakoff (7) in Florida was observed at Sanford, Florida, by I. C. Jagger in 1920 and 1921 and was reported from Tennessee by Sherbakoff in 1921. Takimoto (8) has reported a seed borne bacterial spot disease of peppers in Korea but he isolated a white bacillus entirely different from the yellow monoflagellate form which causes the disease under consideration. Recently the disease has been studied and described in detail by Higgins (6) in Georgia. It occurred near Indianapolis in the fall of 1922 in a crop of peppers grown under overhead irrigation.

In brief it may be said that the disease as it occurs on both hosts is favored by wet weather, that leaf infection occurs through the stomata, that the bacteria are intercellular and the host cells are stimulated toward hyperplasia, particularly in fruit lesions, and that the bacteria are carried on the seed and consequently the disease may be controlled by seed disinfection.

[1] Contribution from the Botanical Department, Purdue University Agricultural Experiment Station, LaFayette, Ind.

[2] The writers wish to acknowledge their indebtedness to Prof. H. S. Jackson for helpful suggestions.

IDENTITY OF THE CAUSAL ORGANISM

Miss Doidge described the causal organism as *Bacterium vesicatorium* n. sp. in her final publication (2) which appeared in February, 1921. We did not have access to this before the publication of our description of the organism as *Bacterium exitiosum* n. sp. in April, 1921 (3). Higgins (6) did not decide upon the nomenclature of the pepper organism. A comparison of the three descriptions shows that all agree that the organism is a monoflagellate, aerobic rod which forms yellow colonies, liquefies gelatin, and produces no gas from carbohydrates. In fact there are only a few really important differences, such as the toleration of acidity and the production of acid from certain carbohydrates.

In order to establish the identity of the organism, a culture (B) of *Bacterium vesicatorium* obtained from Miss Doidge in July, 1921, and a culture (C) of the pepper bacterial spot organism isolated from leaf lesions at Sanford, Florida, on April 21, 1921, by Dr. I. C. Jagger were compared in a series of tests with a culture (A) of our tomato organism. The three strains were found to be essentially similar in appearance and behavior. Cultures on beef agar, potato agar, potato cylinders, and gelatin were similar. In milk with litmus or methylene blue the color was destroyed and the milk was digested without coagulation. All liquefied plain blood serum and Loeffler's blood serum, although the pepper strain (C) failed to produce the brown discoloration noted in the A and B cultures.

All tolerated 3 per cent sodium chloride in neutral beef broth and were inhibited by 4 per cent. The tolerance of acidity was determined by growth in beef broth cultures in which the amount of acid (Fuller's scale) was determined by titration with NaOH with phenolphthalein as an indicator and the hydrogen ion concentration was determined by titration with the sulphone phthalein indicators brom phenol blue, methyl red, brom cresol purple, brom thymol blue, and phenol red.

In terms of Fuller's scale, all strains tolerated HCl up to + 12 and culture C (pepper) endured + 15. All tolerated HCl up to pH 5.0 and were inhibited by a pH of 4.8. All tolerated malic acid up to pH 5.0 but were inhibited by a pH of 4.5. All were inhibited by a pH of 4.8 obtained by adding acetic or citric acid. The three strains grew in broth rendered as alkaline as —25 with NaOH and grew in broth to which NH_4OH was added until a pH of 8.8 was shown with cresol red.

The principal differences in the published descriptions related to the production of acid from carbon compounds. The three strains were grown in test tubes containing 2 per cent solutions of dextrose, saccharose, maltose, lactose, glycerin, and mannit made up in a 2 per cent peptone solution and adjusted to a pH of about 7.0. One series contained the

indicator brom cresol purple, one, brom thymol blue, and the third, phenol red. The three strains behaved similarly and in no case was there any evidence of acid production. There was a marked increase of alkalinity in the mannit and glycerin cultures while the others remained neutral or became slightly more alkaline.

On litmus beef extract agar slants containing 2 per cent dextrose, saccharose, or lactose, all cultures were similar and no acid production was indicated. In cultures on beef extract agar slants containing the three carbohydrates and the hydrogen ion indicators, there was no evidence of acid production and increased alkalinity was indicated by the red color of the phenol red series, especially in the lactose cultures.

Both Miss Doidge and Higgins found ammonia produced by the organism and to this, Higgins ascribes the failure to detect acid production in the standard media. By reducing the protein content and increasing the sugar content of the media he was able to demonstrate acid production from dextrose, saccharose, and lactose.

However, under the conditions of our tests the three strains were practically identical. Furthermore all proved infectious to tomato and with the tomato strain (A) successful inoculation of pepper leaves and fruit was obtained. Therefore it seems safe to conclude that the causal organism of tomato bacterial spot described in Indiana, of tomato canker described by Miss Doidge, and of the bacterial spot of pepper are identical and by priority rules should be known as *Bacterium vesicatorium* Doidge.

TEMPERATURE RELATIONS

Miss Doidge (2) found that the organism grew throughout a wide range of temperatures with an optimum near 30° C. and the writers (3) found likewise except that the optimum lay between 25° and 30° C.

A loopful of a suspension of the tomato organism (A) was placed in the center of each of a series of 16 poured plates of plain potato agar and after three days incubation at room temperature, the colonies were measured. Two plates were then placed in damp chambers in each of a series of eight constant temperature incubators providing a range of temperatures from 2° to 35° C. At the end of 15 days the long and short diameters of the colonies were measured and the mean increase in diameter at 2° was 5.5 mm., at 9°, 8.5 mm., at 16°, 17 mm., at 20°, 18.5 mm., at 23°, 26 mm., at 27°, 31.5 mm., at 30°, 12.5 mm., and at 35°, 10 mm. These results show that the organism grows in a wide range of temperatures with an optimum at 27° C. as is shown graphically in figure 1.

The thermal deathpoint of the pepper organism (C) was found to be 49° C., very near to that previously reported for the tomato organism (49°–50° C.).

EFFECT OF FREEZING

In order to determine the effect of freezing, water suspensions of the tomato organism (A) were made up in five small flasks and frozen by embedding in crushed ice and salt in a container which was stored in a refrigerator. The temperature of the ice-salt mixture was —10° to —12° C. The number of bacteria per cubic centimeter in each flask was estimated by the poured plate method before freezing and at different intervals certain of the flasks were thawed and plate counts were made. Flask 1 was refrozen once, flask 4, twice, and flask 5, four times. While the counts based on the plates are considered only as approximations, the results are presented in table 1. The percentage of viable organisms in terms of the original suspension is included.

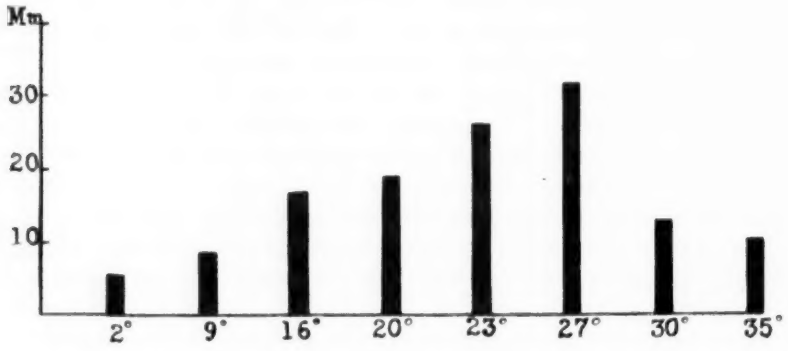


FIG. 1. Effect of temperature on increase in colony diameter of *Bacterium vesicatorium*.

These results show that the mortality increases with the duration of the freezing and with the alternate freezing and thawing. Two to 23 hours of freezing killed 25 to 37 per cent of the organisms, 40 hours, 88 per cent, and 65 hours, 93 per cent. Refreezing 12 hours killed 90 per cent of the organisms remaining alive in flask 1 after the first two hours of freezing, and refreezing six hours killed 56 per cent of the organisms alive in flask 4 after the first 40 hours of freezing. Freezing for eighteen days (flask 5) during which thawing and refreezing occurred four times was sufficient to kill all of the organisms.

It is necessary to admit however that the organism is rather resistant to freezing in water. This is further substantiated by the fact, discussed later, that the organism survived a winter in the field.

HOST RANGE

In our previous report (3) this organism was reported parasitic upon tomato, pepper, and potato. Miss Doidge (2) was unable to infect

TABLE 1—*Effect of freezing in water suspension.*

Time frozen	Flask 1		Flask 2		Flask 3		Flask 4		Flask 5	
	Bacteria per cc.	Per cent	Bacteria per cc.	Per cent	Bacteria per cc.	Per cent	Bacteria per cc.	Per cent	Bacteria per cc.	Per cent
0	2,164,000	100	4,869,000	100	2,770,000	100	2,126,000	100	1,609,000	100
2 hrs.	1,617,000	75								
14 "	165,000	8	2,959,000	61						
23 "					1,742,000	63				
40 "							249,000	12		
46 "							108,000	5		
65 "							28,000	1	105,000	6.5
87 "									78,000	5
8 days									1,800	0.1
16 "									300	0.02
18 "									0	0

lemon seedlings, cotton seedlings, young walnut trees, cabbage, bean, and tobacco seedlings, sweet peas, and *Solanum incanum*. She obtained infection of *Solanum nigrum*, *Physalis minima*, and *Datura stramonium*, var. *tatula*. Higgins (6) reports infection in Pimento, Tabasco, Chili, and bell peppers.

Atomizer inoculation under greenhouse conditions with the tomato organism (A) has yielded only negative results with turnip, cabbage, eggplant, tobacco, *Datura stramonium* L., *Solanum carolinense* L., *S. integrifolium* Poir., and *S. opacum* A. Br. and Bouché. Infection occurred on *Lycopersicum pimpinnellifolium* Dunal (currant tomato), *Lycium halimifolium* Mill. (matrimony vine), *Lycium chinense* Mill., *Hyoscyamus niger* L. (henbane), *Hyoscyamus aureus* L., *Nicotiana rustica* L. (wild tobacco), *Solanum dulcamara* L. (bittersweet), and under field conditions on *Solanum rostratum* Dunal (buffalo bur).

Abundant infection of tomatoes with the pepper organism (C) was obtained in the field as was also abundant infection of peppers, including fruit lesions, with the tomato strain (A). In the greenhouse successful inoculation of *Hyoscyamus niger*, *Hyoscyamus aureus*, and *Nicotiana rustica* was obtained with the pepper strain (C).

With the tomato strain (A) atomizer field inoculations in August, 1921, resulted in infection on 32 seedsmen's tomato varieties not included in our previous list of 41 susceptible varieties and fruit infection occurred in all but four. The Yellow Cherry was found susceptible. No resistant varieties were found.

FRUIT INFECTION

Miss Doidge (2) successfully inoculated unwounded as well as wounded tomato fruits and secured typical scabby lesions when young unwounded fruits were inoculated. In our earlier tests no success attended inoculation of unwounded fruits while infection occurred readily through needle puncture wounds on green fruits but did not result in typical scabby lesions. Higgins (6) reports difficulty in infecting pepper fruits and suggests that there is only a short period early in the development of the fruit when it is susceptible, probably while lenticels are forming. He did not obtain typical scabby lesions from needle puncture inoculations. There is abundant evidence that only young fruits are susceptible.

In our previous account (4) fruit infection through the fruit punctures of stink-bugs and tarnished plant bugs was reported and an opinion was expressed that fruit infection very likely occurs through wounds because of the lack of stomata on tomatoes. The fruit lesions thus obtained were not typical however.

In order to determine the conditions permitting fruit infection and if possible the nature of the white bird's eye spot of tomatoes (Pl. XIV, A, B), two types of insect-proof cages were used in 1921, one of gauze covering the entire plant and the other of celluloid covering a single cluster of fruits. Each cage of the latter type was replaced by a paper bag a few days after it was used.

One to five stink-bugs were caged over each of six clusters of small fruits on July 26 and over each of four clusters on August 4. No inoculum was introduced and upon a total of 23 fruits no bacterial spot nor bird's eye spot developed. Typical stink-bug wounds characterized by a slightly sunken, circular, whitish zone or blotch usually with a black point at the center were abundant on all of the fruits (Pl. XIV, G). Such injuries did not occur on fruits protected from stink-bugs. This type of injury is of common occurrence on tomatoes.

In a parallel series of tests one to eight stink-bugs were caged over each of six clusters of small fruits on July 27 and four clusters on Aug. 4 after the fruits had been atomized with a suspension of the bacteria (A). None of these fruits were over four centimeters in diameter. On Aug. 16, 24 of the 26 fruits showed bacterial spot. There were two types of lesions, one the more typical, small, elevated type (Pl. XIV, D) which develops into a scab, and the other a sunken area of blackened tissue (Pl. XIV, F) about a stink-bug punctures. A section through one of the latter type of lesion is shown in plate XIV, figure E. One bird's eye lesion also developed.

In a third series of tests a number of fruits of various sizes were atomized with a suspension of the bacteria and were caged to exclude all insects. Three fruits between one and 2.5 centimeters in diameter atomized on July 28 showed bacterial spot infection on Aug. 4, which developed into typical scabby lesions. Eight fruits between 0.6 and 2.5 centimeters in diameter, in three clusters, were atomized on Aug. 4 and by Aug. 16 all showed numerous typical lesions of the elevated type and four showed a few bird's eye spots. A number of fruits varying in diameter from 0.6 to 7.5 centimeters on a plant under a large cage were atomized on Aug. 12. Two weeks later typical lesions, in some cases in large numbers, developed on 15 of the 38 fruits atomized and only on fruits that were not more than 2.5 centimeters in diameter when inoculated, of which there were only 17. Therefore 15 out of 17, or 88 per cent, of the fruits atomized when not more than 2.5 centimeters in diameter developed the disease. No lesions developed on six uninoculated fruits held as checks.

Among seven clusters of fruit caged without insects and without inoculation, none developed bacterial spot nor bird's eye spot.

These results show that bacterial spot infection may occur through stink-bug punctures but that typical scabby lesions result from direct infection of very small fruits (diameter less than four centimeters) without insect punctures. The nature of bird's eye spot is not yet proved but it may be a form of bacterial spot.

Since no stomata have been noted on young fruits, the portal of infection is unknown. Evidence of infection through epidermal rifts about the stem end of the fruit has been noted (Pl. XIV, C). Young fruits are thickly covered with club-shaped glandular hairs and long trichomes and as the fruit enlarges these become fewer and more scattered. On old fruits most of these appendages have disappeared leaving only their broken bases. It seems entirely possible that the bacteria may enter through the wounds caused by the breaking off of these delicate epidermal appendages. Groth (5, p. 5) has described minute cuticular cracks in the epidermis through which it also seems possible that the bacteria might enter.

OVERWINTERING

In our previous accounts (3, 4), proof was presented that the organism overwinters on the seed. In fact the organism was found alive on commercial seed 16 months old and had survived 11 months of desiccation on tomato seed in the laboratory. Subsequent cultural tests have shown some of the organisms to be alive on this seed after 16½ months but none after 35 months.

Evidence of overwintering in the field has been obtained. In 1921 a number of volunteer tomato plants came up in a corn field at LaFayette, which had been in tomatoes in 1920. No tomatoes were planted anywhere near this field in 1921. On July 27, 186 of the volunteer tomato plants were examined and two showed bacterial spot infection. Since the disease had been present in this field the year before, this seems to be clear-cut evidence of overwintering of the organism in the soil or in remnants of diseased plants. Consequently, as Miss Doidge (2) has suggested, crop rotation is advisable.

TOMATO SEED DISINFECTION

Seed disinfection in mercuric chloride, 1 to 3,000, for five minutes followed by rinsing has been found effective and practicable as a control measure. For the 1921 crop in Indiana, 1500 pounds of seed were thus treated and, so far as it was possible to determine, there was very little of the disease in the crop, although an absolute control was not obtained. On the other hand the disease was found very prevalent as early as July 13 in certain fields in which the plants were grown from untreated seed. Observations in a canning factory in 1922 on the crop grown from treated seed showed that bacterial spot occurred only very rarely.

SUMMARY

The causal organism of bacterial spot of tomato and pepper should be known as *Bacterium vesicatorium* Doidge.

The optimum temperature for colony growth is near 27° C.

The bacteria endure short periods of freezing in water but are rapidly killed by several days exposure and by alternate thawing and freezing.

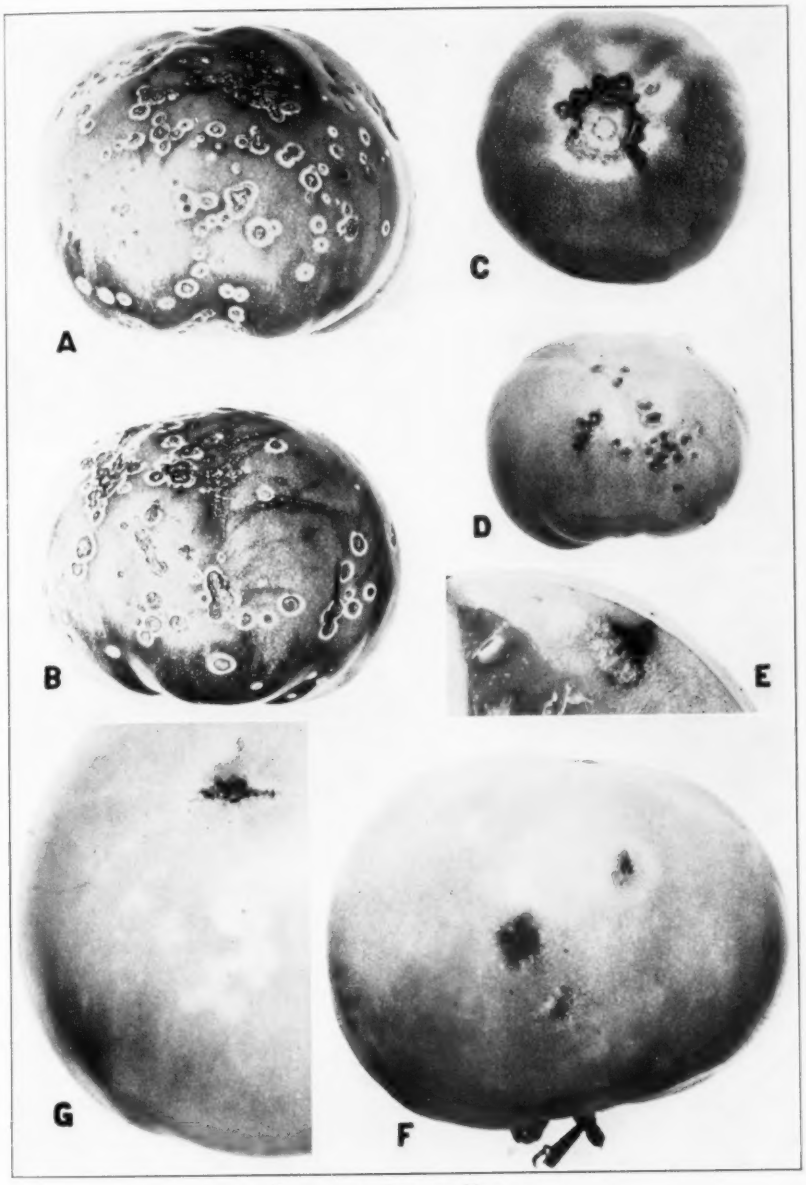
The hosts include tomato (73 varieties), pepper (4 types), potato (leaves), currant tomato, *Solanum nigrum*, *S. dulcamara*, *S. rostratum*, *Datura tatula*, *Physalis minima*, *Lycium chinense*, *L. halimifolium*, *Hyoscyamus aureus*, *H. niger*, and *Nicotiana rustica*.

Fruit infection of tomato may occur through stink-bug punctures but the resultant lesions are not typical.

Typical fruit lesions result from direct infection of very small uninjured fruit.

The organism survived a winter in the field and survived 16½ months desiccation on tomato seed.

Tomato seed disinfection has proved effective and practicable as a control measure.



BACTERIAL SPOT OF TOMATO

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DESCRIPTION OF PLATE XIV

Bacterial Spot of Tomato—A. and B. Birds-eye spot of tomato. Cause not proved. Possibly a form of bacterial spot. C. Bacterial spot lesions about stem end of a tomato possibly resulting from infection of small rifts in the cuticle. D. Early stage of bacterial spot. Typical elevated lesions not resulting from infection of insect punctures. E. Section through a stink-bug wound infected with bacterial spot. F. Bacterial spot lesions resulting from infection of stink-bug wounds. G. Stink-bug injury on tomato fruit, not infected. Circular whitish blotches often with a black point at the center.

THE GAMMA STRAIN OF COLLETOTRICHUM LINDEMUTHIANUM (SACC. ET MAGN.) B. ET C.

WALTER H. BURKHOLDER

In 1919 Dr. M. F. Barrus (2) listed over 300 varieties of beans (*Phaseolus vulgaris* L.) and related species, and gave their variation in susceptibility to *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C., the fungus causing the anthracnose of this plant. In conducting these inoculation experiments, Barrus gathered together a number of cultures of the pathogene from various sources. These cultures he was able to separate into two groups according to the susceptiblity or resistance of certain varieties of beans to them. Two biological strains, therefore, were considered to exist in the species *C. lindemuthianum*. These were designated as alpha and beta. Further isolations of the pathogene made by the writer while working at the Perry Bean Laboratory[1] revealed no other forms. Thus, while it was thought that other strains of this fungus might exist, they were considered rare, and at least in New York State, to be of no importance.

The great majority of the varieties of beans tested by Barrus were susceptible to one or the other of the strains, and many were susceptible to both. A few varieties, however, were resistant to the two strains. Of this latter group the two most important varieties were the Well's Red Kidney and the White Imperial.

The Well's Red Kidney which was brought to the attention of Plant Pathologist by Barrus (1) is immune to strain alpha and highly resistant to strain beta. Since its introduction, it has been grown extensively in New York, and also outside the State. During this time no authentic case has been investigated in which this variety has succumbed to the anthracnose. This appeared to be further evidence of the scarcity of other strains of the pathogene. The White Imperial, the other variety of beans mentioned as resistant to both strains of the anthracnose fungus is resistant to alpha and practically immune to beta. Nevertheless it is not being grown to as great an extent in New York State as the Wells' Red Kidney on account of its lateness which frequently causes it to be prematurely killed by early frosts. Until 1921 this bean likewise continued resistant to the anthracnose as far as inoculation experiments with alpha and beta were concerned, and as far as observations

[1] A laboratory established in 1917 at Perry, New York, for the New York State Bean Investigation, and continued until 1920.

in the field had gone. In that year Barrus (3) writing in regard to the White Imperial states "recently, however, (in 1921) a field of beans said to be White Imperial, the pods of which were severely affected with anthracnose, was observed. It seems not unlikely that strains of the pathogene exist which are capable of infecting even these varieties." No further investigation was made by Barrus of this case, but the address of the grower of the field of White Imperial beans was given to the writer. A quantity of seed was obtained from this field, and in type, it was without doubt a White Imperial. Examination also showed small spots on some of the seeds which appeared to be due to the anthracnose.

During the latter part of January, 1922, petri dish plantings were made from these lesions and a fungus was isolated that proved to be *C. lindemuthianum*. Preliminary inoculation experiments were immediately conducted with this fungus to determine its identity. To obtain spores for these experiments the fungus was grown on bean plugs where it produced great masses of pink spores.[1] Four well-known commercial varieties of beans were used. They were the Wells' Red Kidney, the White Imperial, the White Marrow and the Michigan Robust pea bean. These were selected with the purpose of determining whether the recently isolated fungus was either alpha or beta. It was known that the first two varieties were resistant to both strains of the pathogene, the third variety was resistant to alpha, and the fourth to beta. Of these four varieties only the Michigan Robust proved resistant to the fungus under consideration, so that no definite clue was given to its identity. The Wells' Red Kidney which hitherto had remained resistant to all inoculations of *C. lindemuthianum* was especially susceptible to this fungus. Young seedlings not only showed lesions due to the disease, but were killed within a few days after the symptoms began to appear.

Inasmuch as the Michigan Robust was resistant to the new strain and also to beta, there was the possibility that we were dealing with a vigorous strain of the latter. During the summer of 1919, however, a very virulent strain of beta was isolated from a pod of a *Nova Scotia Marrow*[2] and tested on 35 varieties of beans. The White Imperial and

[1] This appears to be the usual case with the anthracnose fungus when recently isolated. When grown in culture for a considerable period, a year or even less, the fungus loses this power of fructification to a great extent. Re-isolation, however, will restore this ability and is the only satisfactory method found by the writer for this purpose, although various methods have been proposed by other investigators. This loss of virulence in the pathogene probably varies with the strains since more trouble has been experienced in keeping alpha in a good sporulating condition than with beta.

[2] A type of White Marrow grown in Nova Scotia, and brought to the attention of the writer by Mr. G. E. Sanders. Under field conditions in New York State it is more or less resistant to the anthracnose.

Wells' Red Kidney remained very resistant to the Nova Scotia strain although varieties susceptible to beta succumbed very rapidly.

To determine further, whether or not the fungus isolated from the White Imperial was a new strain of *C. lindmuthianum*, more than sixty varieties of beans were obtained for inoculation experiments. These experiments were conducted during the spring and summer of 1922 in the following manner. The varieties to be tested were planted in flats in the greenhouse in rows of 25 to 30 individuals. Inoculations were made as soon as possible after germination by spraying the plants with a water suspension of conidia The flats were then placed in an inclosed chamber, the temperature of which was approximately 18° C. After two days in this chamber the plants were removed and placed in a greenhouse with a temperature of 26° C., or above. Here, at the end of five days or more lesion began to appear. Lauritizen (5) has found that with the anthracnose of the bean very little infection occurs at 27° C., but there is a rapid increase with the lowering of the temperature; excellent infection being obtained as low as 17° C. But the writer is of the opinion that infection having once taken place, the pathogene advances in the tissues more rapidly at higher temperatures. The incubation period is shortened, and the lesions, too, develop to a greater extent.

In all sets of inoculation experiments certain varieties of beans showed excellent infection. The results of these experiments are to be found in table 1. Here, in describing the degree of susceptibility of the varieties of beans to this strain of the pathogene the terminology of Barrus (2) is followed. In his article, these various terms are illustrated with photographs. If table 1 is compared with Barrus's work, it will be seen that the pathogene isolated from the White Imperial agrees very closely with the beta strain of *C. lindemuthianum*. It differs mainly in four varieties. With the Wells' Red Kidney and White Imperial this difference is very pronounced. With the Arlington Red Cranberry and the White Sickle it is less striking, but still evident.

Since the recently isolated strain was so similar in its behavior to beta, further inoculation experiments were desirable before deciding whether or not it was a new strain. There was also the possibility that the seed of the Well's Red Kidney and the White Imperial used in the above experiments varied from the original stock, and for that reason were susceptible. Therefore, 26 strains of the Wells' Red Kidney, 4 strains of the White Imperial, 12 strains of the Yellow Eye, and one each of the Arlington Red Cranberry and the White Sickle were collected. All strains of the Wells' Red Kidney and the Yellow Eye. and two of the

TABLE 1—*Inoculation experiments with the strain of Colletotrichum lindemuthianum isolated from the White Imperial bean.*

	Variety	Infection
1	Arlington Red Cranberry	good
2	Best of All	fair
3	Black Valentine	fair
4	Black Valentine	fair
5	Canadian Wonder	good
6	California Pink	very slight
7	Case Knife	none
8	Cracker jack Wax	good
9	Crease Back	none
10	Crystal Wax	slight
11	Curries	excellent
12	Currie Improved Prolific Tree	none
13	Davis Wax	excellent
14	Detroit Wax	good
15	Eureka	excellent
16	Flat Marrow	slight
17	French Mohawk	good
18	Full Measure	good
19	German Black Wax	excellent
20	Giant Stringless	excellent
21	Golden Eye Wax	good
22	Hardy Wax	slight
23	Hodson	good
24	Isabelle Improved Michigan Pea	none
25	Kentucky Wonder	none
26	Kentucky Wonder Wax	none
27	Lazy Wife	good
28	Longfellow	good
29	Low Champion	good
30	Michigan Robust Pea	none
31	Mont d'Or	excellent
32	Nova Scotia White Marrow	good
33	Old Fashioned Sickle	slight
34	Pencil Pods Black Wax	excellent
35	Pheasant Eye	excellent
36	Prolific Black Wax	excellent
37	Red Kidney (Thorburn)	excellent
38	Red Kidney	excellent
39	Refugee	excellent
40	Refugee Wax	slight
41	Refugee Wax	slight
42	Ruby Horticultural	excellent
43	Scotia	very slight
44	Scotia	none
45	Souther Prolific	none
46	Sure Crop	slight
47	Tennessee Green Pod	none

Variety	Infection
48 Turtle Soup	none
49 Unrivalled Dwarf Wax	excellent
50 Warren	fair
51 Wardwell	excellent
52 Webber Wax	excellent
53 Wells' Red Kidney	excellent
54 White Imperial	excellent
55 White Kidney	excellent
56 White Marrow	excellent
57 White Navy (Burpee)	none
58 White Navy (Harris)	none
59 White Sickle	very slight
60 White Sickle	none
61 Wocester Mammoth	good
62 Yellow Eye	slight
63 Yosemite	excellent

White Imperial were obtained from Mr. F. R. Perry of the Hicox-Rumsey Seed Company of Batavia, New York. The Yellow Eyes were originally from Dr. H. H. Love of the Department of Plant Breeding at Cornell, and were pure line strains.

In conducting the inoculation experiments both beta and the newly isolated strain were used. The beta strain came originally from Barrus, and excellent infection had been obtained when susceptible varieties of beans were inoculated with it. Nevertheless, to make this culture more comparable with the new strain, some White Marrows were infected and a re-isolation made. This re-isolated culture was then used in the following experiments. The inoculations were conducted in the same manner as previously described. Corresponding flats were inoculated at the same time with beta and the new strain. The data are set forth in table 2.

With the Wells' Red Kidney it will be noted that the new strain gave excellent infection in the majority of cases, good in a lesser number, fair in two cases, and slight in one. On the other hand, the beta strain caused no infection in the majority of cases, slight in one, fair in two, good in one, and excellent in one. One significant point in this experiment-test should be noticed, and that is, that those strains showing a slight susceptibility to beta frequently exhibit, a slight resistance to the White Imperial strain. This is the condition in numbers, 3, 7, 12, 18, and 21. It cannot be argued then, that the new strain is only a more virulent form of beta. In the Yellow Eye, too, it may be observed that some of the pure lines are more resistant to the new strain than to beta. As in the majority of Barrus's inoculations the Arlington Red Cranberry was resistant to the beta strain, but excellent infection was

TABLE 2—*Inoculation experiments with the beta strain and the White Imperial strain of C. lindemuthianum.*

No.[1]	Infection	
Wells' Red Kidney	Beta Strain	White Imperial strain
2	none	excellent
3	slight	good
4	none	excellent
5	none	excellent
7	fair	good
8	none	excellent
9	none	excellent
10	none	excellent
11	none	excellent
12	good	slight
13	none	excellent
14	none	excellent
17	none	excellent
18	fair	good
21	excellent	good
29	none	excellent
31	none	excellent
32	none	fair
37	none	good
38	none	excellent
51	none	excellent
52	none	excellent
53	none	fair
54	none	good
55	none	excellent
56	none	good
White Imperial		
1	none	good
2	none	good
3	none	good
4	none	good
Yellow Eye		
2-7-28-30-7	slight	none
5-68-45-1-3	slight	none
17-541-8-5-3	good	fair
17-541-8-5-44	good	slight
17-541-12-1-6	fair	none
20-560-1-3-1	slight	fair
20-560-1-3-11	slight	fair
26-474-6-5-12	slight	fair
26-474-6-5-27	slight	fair
26-478-27-13-6	slight	fair
26-478-27-13-22	slight	fair
26-478-29-20-8	slight	fair
26-478-29-20-25	slight	fair
Arlington Red Cranberry	very slight	excellent
White Sickle	very slight	none

[1] The pedigree numbers of the Wells' Red Kidney and the Yellow Eye are those used by Mr. Perry and Dr. Love.

obtained on this variety in the experiments being reported upon, with the White Imperial fungus. This fact also seems to indicate that the recently isolated fungus is a new strain. On the other hand, the White Sickle was resistant to both pathogenes and was evidently a different strain of that variety of bean from the one used by Barrus in his experiments.

Much of the work done at the Perry Bean Laboratory to control the anthracnose has been in breeding strains of beans resistant to the disease. Dr. G. P. McRostie (6 and 7) and the writer (4) have shown that resistance to alpha and beta respectively is dominant and governed by a single factor. Just how the resistant to the third strain of *C. lindemuthianum* would react in hybridization was an important point to be considered in further breeding experiments. A small number of seed from the F_1 plants of crosses between the Wells' Red Kidney and the Michigan Robust pea bean were on hand. These were planted in flats and inoculated with the third strain. The data which were collected and tabulated on July 26, 1922 may be found in table 3. The number of F_2

TABLE 3.—*Results of the inoculation of F_2 plants of a cross between Wells' Red Kidney and Michigan Robust pea bean with the White Imperial strain of C. lindemuthianum.*

Pedigree number	Resistant	Susceptible
402	82	23
403	57	23
404	9	2
405	34	8
406	27	9
Totals	209	65
Expected number	204	68

individuals is small, but with the close approximation to a three to one ratio, there seems to be no doubt that resistance is dominant and governed by a single factor. In this respect it agrees with the resistance to both alpha and beta. It should be observed that in this experiment the Wells' Red Kidney was used as the susceptible parent. In the hybridization experiments previously recorded, Burkholder (4) and McRostie (6 and 7), this variety had always been used as the resistant parent.

From the foregoing experiments the strain of *C. lindemuthianum* isolated from the White Imperial appears to be distinct from either alpha or beta. It is true that it is closely related to the latter, but the inoculation experiments on the Wells' Red Kidney and the Yellow Eye show that it is not merely a more virulent form. The hybridization experiments too, lend proof to this point since the susceptible parent in the experiment was resistant to the beta strain of the pathogene.

In speculating as to the origin of this new strain, the writer considers it is not improbable that it arose from the beta strain as a mutation. The close relationship of the two strains as demonstrated in table 1 points toward this possibility. It is also known that the beta strain under favorable circumstances will produce, at times, fair sized lesions on young seedlings of the Wells' Red Kidney, so the further step to the new strain would not be great. Such a case not unlikely was the beginning of the third strain. Moreover, the county in which this new strain was found, is one in which the Wells' Red Kidney is grown extensively, and under these circumstances chance would be given for its development. All things considered, however, the writer feels justified in regarding the fungus isolated from the White Imperial sufficiently removed from beta to be considered a new strain of *C. lindemuthianum*, and for this the Greek letter *gamma* is given to correspond with the alpha, and beta strains.

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TRYPANOSOME-LIKE BODIES IN SOLANACEOUS PLANTS

J. E. Kotila and G. H. Coons

Immediately following the recent report by Nelson (1) of the occurrence of protozoa in bean, clover, and tomato plants affected with mosaic and potato plants affected with leaf roll, investigations were undertaken to ascertain whether the same conditions obtained in potato plants affected with other degenerational diseases.

Diseased material from plants showing severe symptoms of potato mosaic, streak and leaf roll was killed and fixed with both chrome-acetic and Zenker's solutions. Longitudinal sections seven microns thick were cut and stained with Haidenhain's haematoxylin, the sections being mordanted 4 to 6 hours, stained for 12 hours and restained until most of the haematoxylin had been removed from the cell nuclei. The destaining process seldom required more than two minutes in the 4 per cent iron alum mordant which was used.

Cell inclusions resembling the trypanosome-like bodies described by Nelson were found in phloem cells of potato plants affected with mosaic and streak. Likewise, similar bodies were found in the sections from plants affected with leaf roll.

Trypanosome-like bodies having been found in the material from the potato plants affected with the various diseases, the studies were extended to other Solanaceous hosts affected with mosaic. Midribs and lateral veins of leaves of mosaic tobacco and petunia plants were killed, fixed and stained as described for potato. No difficulty was experienced in locating in the phloem cells, inclusions similar to those found in the diseased potato plants.

As a control upon the study of diseased material, similar work was done with material from healthy potatoes and tomatoes. The stock from which the healthy potato material was taken was indexed[1] during the winter of 1921–1922, found to be healthy, and planted in an isolated plot in a sunflower field during the 1922 growing season. No mosaic or leaf roll appeared in the plot during that summer. Some of the progeny of this healthy stock was indexed again during the present winter, it being planted in a greenhouse in which indexing of potatoes was being carried on. Aphids were extremely scarce on the potatoes in the greenhouse and none were observed on the plants which were selected for study. No signs of either mosaic or leaf roll were observed on any of the plants which had reached the height of eight inches at

[1] In this work, an eye of a tuber was sprouted and grown in the greenhouse to approximately 10 inches in height, and evidences of disease looked for. The tubers from which any aberrant plants developed were rejected.

the time material was cut and fixed. The stock of potatoes studied was of the Bliss Triumph variety, upon which symptoms of either mosaic or leaf roll can easily and promptly be recognized.

The tomato material was obtained by planting disinfected (chlorazine 1 per cent) seed on agar in petri dishes, and after germination by transferring the seedlings to test tubes containing Shive's nutrient agar. Material was taken for study when the seedlings were approximately one and a half inches tall. The test tubes containing the seedlings were kept in test tube racks in a window of the laboratory and being plugged with cotton there was no possibility of contamination by aphids. The only possible source of disease was in the seed itself. Allard (2) and Gardner and Kendrick (3) contend that tomato mosaic is not transmitted through seed and base their contentions on negative results obtained from planting seed and growing thousands of tomato plants under controlled conditions. Gardner and Kendrick (3) state "In the light of all the available evidence, there appears to be no indication whatever that tomato mosaic is transferred through the seed."

It is believed that this material studied was healthy. Stained sections from both potato and tomato plants revealed cell inclusions in the phloem elements which were similar in size and form to those found in severely diseased material and which were equally abundant.

Although the cell inclusions observed were trypanosome-like in shape, they did not possess any degree of uniformity. Great variation in size was found in the bodies from both diesased and healthy material. All attempts to show definite structure by using such protozoan stains as Wright's, Romanowski's, tetrachrome, etc., have resulted in failure, the bodies staining a uniform blue. Likewise it has been impossible to demonstrate motility by means of thin sections of fresh material from either diseased or healthy plants or in extracted juice of such plants

From the study which has been made of both diseased and healthy plants it is believed that the correlation of the trypanosome-like bodies described by Nelson with mosaic and leaf roll has not been proved and further investigations are necessary.

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MARCH 1923.

INTRACELLULAR BODIES IN THE PHLOEM TISSUE OF CERTAIN PLANTS AND THEIR BEARING ON THE MOSAIC PROBLEM

S. P. Doolittle and H. H. McKinney

With Plate XV

In connection with investigations of mosaic diseases the writers have studied large amounts of phloem tissue of both mosaic and mosaic-free plants.

Studies have been made of stem and petiole phloem elements of mosaic-free and mosaic-infected navy beans, tomatoes, cucumbers, and red clover and of healthy garden peas, sweet peas, and alfalfa. In most cases free-hand razor sections of fresh material were studied in comparison with material fixed, embedded, sectioned, and stained by the usual methods. In these studies certain intracellular bodies have been found in the phloem of both mosaic and mosaic-free plants. In view of the very close similarity of these bodies to those which Nelson (2, 3) recently described as protozoa in the phloem of certain mosaic plants, it seems desirable to publish some of our recent results.

The mosaic-free bean and tomato plants studied by the writers were grown in an isolated greenhouse at the University of Wisconsin, in which no mosaic plants were found, either before or after the time the material for study was taken, even though light and temperature conditions were favorable for the development of mosaic symptoms. In fact, no mosaic-infected tomato plants were found in any of the University greenhouses. Furthermore, healthy tomato plants inoculated with juice from the healthy plants sectioned developed no mosaic, while similar healthy plants inoculated at the same time from the mosaic plants studied, all developed the disease. The mosaic-free beans used were grown from seed of both mosaic-resistant and mosaic-susceptible varieties taken from mosaic-free plants.[1]

The mosaic-free cucumbers were grown at Madison, Wis., under cheesecloth cages, which have proven effective in preventing mosaic infection by excluding the insect carriers. The mosaic-free red clover plants and healthy garden peas, sweet peas, and alfalfa were grown in the University greenhouses from healthy seed.

[1] The writers are indebted to Mr. W. J. Evans, graduate student in the Department of Plant Pathology, University of Wisconsin, for bean seed and plants with which he was working in connection with his mosaic resistance studies. They are also indebted to Dr. F. R. Jones and Mr. John Monteith, Jr., for pea and clover material, respectively.

The mosaic-infected tomato plants were obtained in a commercial greenhouse in South Madison, Wis. The mosaic-infected bean and clover plants were grown in the University greenhouses at Madison. The mosaic-infected cucumbers studied were grown in fields and greenhouses at Madison.

In mosaic-free and mosaic-infected bean plants, three to six weeks old, the bodies were found both in unstained, freehand razor sections of fresh material cut longitudinally and in stained, longitudinal sections cut 9–12 microns thick from material killed in various fixatives, embedded and stained either by Flemming's triple stain or by Heidenhain's iron-alum haematoxylin. The bodies were found singly in the sieve-tube and adjoining cells oriented longitudinally. They were of various shapes from irregularly ovoid to narrowly ellipsoid and usually had one or more slender strands extending from each end. In some cases, these strands extended to the ends of the cells, and in others, limited distances from the bodies (Pl. XV–B). While the general form of some of these bodies may suggest that of certain protozoa, the characteristic structural details of protozoa, such as nuclei, blepharoplasts and rhizoplasts, were not found when the material was stained with Heidenhain's iron-alum haematoxylin and carefully destained, or when stained with Flemming's triple either of which stains should show these internal structures if present. In free-hand razor sections of fresh material, no active motion of the intracellular bodies was noted, such as might suggest that of a protozoan.

In mosaic-free and mosaic-infected red clover, as well as in healthy garden peas, sweet peas and alfalfa, intracellular bodies have been found which, in general, are similar to those found in the bean, except that the terminal strands have been noted only occasionally in clover and alfalfa and not at all in garden peas or sweet peas.

While the writers have not yet made an exhaustive search for literature bearing on these intracellular bodies, it is of especial interest to note that in 1891 Strasburger (4) reported finding, in presumably healthy plants of black locust (*Robinia pseudoacacia*), certain bodies which resemble, in many respects, the bodies found by the writers in beans, clover, garden peas, and sweet peas (Pl. XV-A).

The pertinent portion of the Strasburger (4) article has been translated as follows:

"The mature sieve-tube cells contain ellipsoidal bodies (Pl. III, Fig. 4).[1]

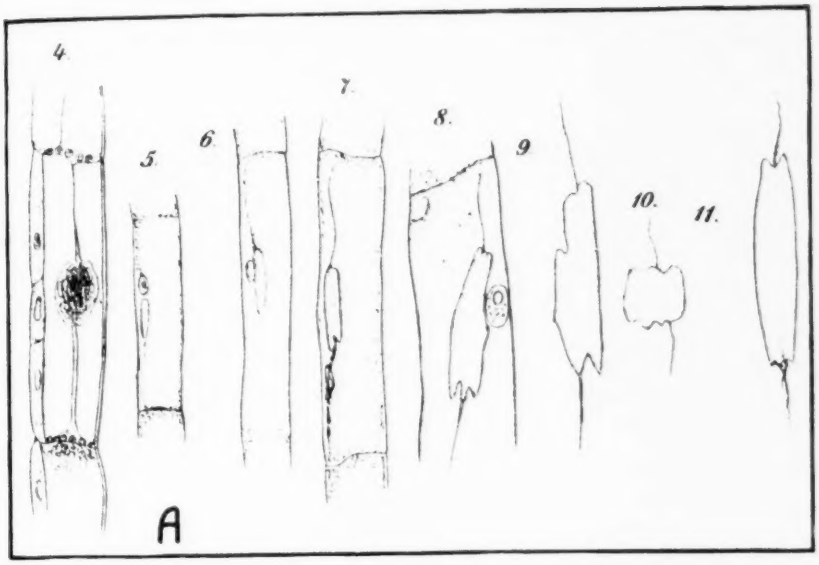
In an alcoholic preparation there is to be seen in each element a strongly refractive lump which gives the customary reaction of the sieve-tube slime, but which generally appears to be suspended in the middle of the cell. The suspension occurs by means

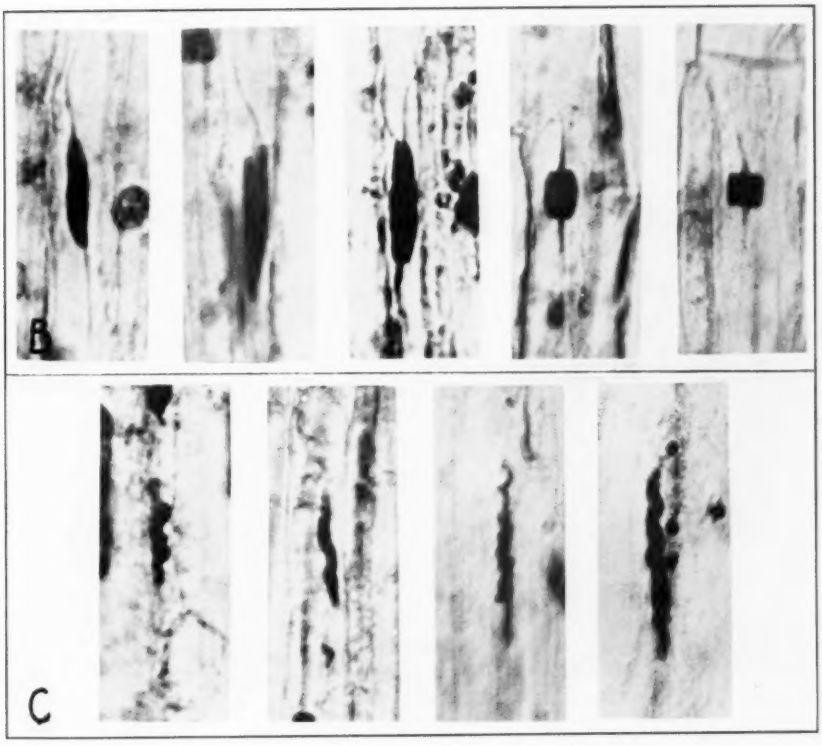
[1] Strasburger's Plate III, figs. 4–11 are reproduced in our plate——A.

of thin threads which lead from ends of the slime-body toward the sieve-plates. This thread becomes increasingly attenuated until it reaches the sieve plate, where, however, it often widens. If the slime body lies against one or the other sieve-plate, it sends a single thread to the other sieve-plate. The suspended slime-body has a somewhat ellipsoidal or angular polygonal shape. * * * The slime-body colors a yellow-brown with iodine although the thread takes the same color, only exceedingly faintly. The slime-body similarly reacts intensively in Millon's reagent and the threads weakly. Ontogeny shows that the bodies arise from the primordial protoplasm of the cell. It shows at the time of its formation, chiefly a spindle (Pl. III, Fig. 5–7), seldom a barrel-shaped (Pl. III, Fig. 10) form. As soon as the slime body reaches a certain size the thread leading from the body to the sieve-plates becomes visible. This thread also is differentiated from the inner portion of the peripheral protoplasmic layer. The slime-bodies swell into an ellipsoidal form (Pl. III, Fig. 7–9), and together with their suspension threads, pull away from the peripheral layer of protoplasm. In the meantime the nucleus shrinks and the protoplasmic layer is very decidedly reduced. The protoplasm contracts in the young sieve-tubes but in old ones fails to contract and is therefore not always readily demonstrable. It often happens that more than one suspension thread may lead from the same end of a slime-body to one or the other of the sieve-plates. Less often the slime-body arises in contact with the sieve-plate. One finds them commonly at the sieve-plates but this is usually a result of the cutting of the stem sections and the influence of the alcohol. That the suspended slime-body cannot be considered an artifact is easily demonstrated in fresh material in which the somewhat refractive slime-body as well as its threads can be readily recognized."

Haberlandt (1) states that these slime-bodies occur generally in the Leguminosae but do not occur in the Cucurbitaceae. Recent as well as earlier studies by one of the writers (Doolittle) are in accord with this statement, as hundreds of longitudinal sections made from both mosaic and mosaic-free cucurbit stems and petioles have never revealed such bodies in the phloem tissues.

In the case of the tomato, the intracellular bodies, interpreted by Nelson as trypanosome-like protozoa, apparently do not occur as regularly as the bodies which are found in the bean, but they seem to be fully as abundant in the mosaic-free as in the mosaic tissues (Pl. XV-C). Careful staining and destaining of these bodies by the methods used in studying the bodies in the bean has failed to reveal structure typical of trypanosomes or other protozoa. Examinations of the tissues of both mosaic and mosaic-free plants show that the cytoplasm of the phloem cells frequently collects in lightly stained, long or short, irregular, spiral, wavy forms and it is possible to find masses which show all stages between this cytoplasmic condition and the deeply stained forms which seem to be identical with the bodies described as protozoa by Nelson. The writers have looked for a possible undulating membrane, a characteristic of the trypanosomes, but none has been found. In some cases the outer edges stain lighter than the central portion of the bodies, but this could easily be due to wrinkled or folded host cytoplasm.





INTRACELLULAR BODIES OF MOSAIC-FREE PLANTS

While it is recognized that Strasburger might have been working with mosaic plants, this seems improbable. The possibility of infection in the case of the plants which the writers have considered mosaic-free is extremely slight, as they were grown under conditions which assured unusual protection from infection by means of insects or other agencies and which would not favor a masking of symptoms.

As the bodies described by Nelson in beans, clover, and tomato have been found by the writers in mosaic-free plants,[1] and as they cannot be distinguished from what seem to be normal constituents of the cells, further work will be necessary before it can be said definitely that protozoa are present in plants affected by mosaic diseases.

[1] Since preparing this manuscript, Dr. E. A. Bessey, of the Department of Botany, Michigan Agricultural College, who is familiar with Nelson's material, has seen the material studied by the writers and has stated that the bodies with which they are working appear to be identical with those described by Nelson.

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LEGENDS FOR PLATE XV

A. Copy of figs. 4–11 from Pl. III of Strasburger paper (4), a portion of which is translated in text, showing slime-bodies in the sieve-tubes of *Robinia pseudoacacia*. Translation of original legends:

Fig. 4. Mature sieve tube elements with companion cells; in the interior of the sieve-tube the slime-body suspended on a thread. (× 240.)

Figs. 5, 6, & 7. Young sieve-tube elements which show the ontogeny of the slime-body in the peripheral protoplasmic layer. (× 240.)

Figs. 9. 10, & 11. Slime-bodies more highly magnified. (× 540.)

B. Various bodies found by the writers in the sieve-tubes of mosaic-free navy bean plants. (× 1144.)

C. Various bodies found in the phloem-tissue of mosaic-free tomato plants. (× 1144.)

NELSON'S SPIRAL BODIES IN TOMATO MOSAIC NOT PROTOZOANS

CHARLES A. KOFOID, HENRY H. P. SEVERIN AND OLIVE SWEZY

Since the publication of Nelson's account[1] of his evidence for the occurrence in certain mosaic diseases of plants of protozoans resembling trypanosomes, we have been able to examine both normal tomato plants and those with typical mosaic. These plant tissues we fixed in hot Schaudinn's fluid and stained with the refinements of protozoological technique in iron haematoxylin. We find structures in both the normal and diseased tissues thus prepared which belong clearly to the same category as the structures described and figured by Nelson. We are wholly unable to agree that these are trypanosomes and find nothing convincing that they are of a protozoan nature.

They are not trypanosomes, for they have no undulating membrane, no flagellum with a definite, clear-cut, marginal fibril arising from a centrosome. Neither is there a parabasal body, the so-called kinetonucleus of earlier literature and textbooks, near the end of the fibril and joined to the centrosome by the parabasal rhizoplast. Furthermore, we are unable to agree that the spiral form of these bodies, which are often twisted in a total spiral, is characteristic either of trypanosomes or of any known protozoans. Its modifications are too strangely varied to fall into any known category of the morphological aspects of the protozoa. In addition, we can not agree that a nucleus of flagellate type with a nuclear membrane, a karyosome, and peripheral chromatin, such as might be expected in an organism with trypanosome affinities, or, indeed, of any known protozoan type, is present in these bodies. Nelson's figures do not demonstrate it to us and we can not agree that the darker aggregates sometimes demonstrable in the spiral bodies have either the constancy of structure, the type of variability, or the evidences of mitosis to be found in protozoan nuclei.

These spiral bodies of tomato mosaic do not belong in the same category with the clearly established trypanosomes of the latex of Euphorbiaceous plants, which bear all the morphological tokens of their protozoan and trypanosome affinities.

We did not see at any time any evidence of motility on the part of these spiral bodies, when observed in the expressed juices of the tomato plant. There was no movement of an undulating nature, no lashing

[1] Nelson, Ray The occurrence of protozoa in plants affected with mosaic and related diseases. Mich. Agr. Exp. Sta. Tech. Bul. **58**: 1–30, *18 figs.* 1922.

of a flagellum, no darting across the field of the microscope such as characterize the activities of motile flagellates. Occasionally reagent bottles, notably those containnig physiological salt solution, which are in use in the laboratory, become contaminated with a species of *Bodo*, a small, rapidly-moving flagellate. These might appear in preparations made up with such contaminated solutions and deceive the observer as to their origin.

Finally, we find the same, or at least similar structures in comparable locations in supposedly healthy and evidently mosaic-free seedling tomato plants, as well as in the diseased phloem of older plants. The only difference noted between the spiral bodies from the diseased and supposedly healthy plants is that the former are larger in most instances observed in our material. We are unable to state whether or not this difference bears any relation to either age or to disease of the plant. This occurrence of the spiral bodies in both supposedly healthy and obviously diseased plants adds to our skepticism as to their protozoan affinities and as to the possibility that they are flagellates from the digestive tract of plant feeding Hemiptera. The peculiar relations of these spiral structures in their elongated, trumpet-shaped phase to the sieve plates in some instances, their relative homogeneity, and their spiral structure, lead us to believe that they are dexiotropic cell contents of an albuminoid nature.

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SLIME BODIES OF ROBINIA PSEUDO-ACACIA L.

IRVING W. BAILEY

WITH PLATE XVI

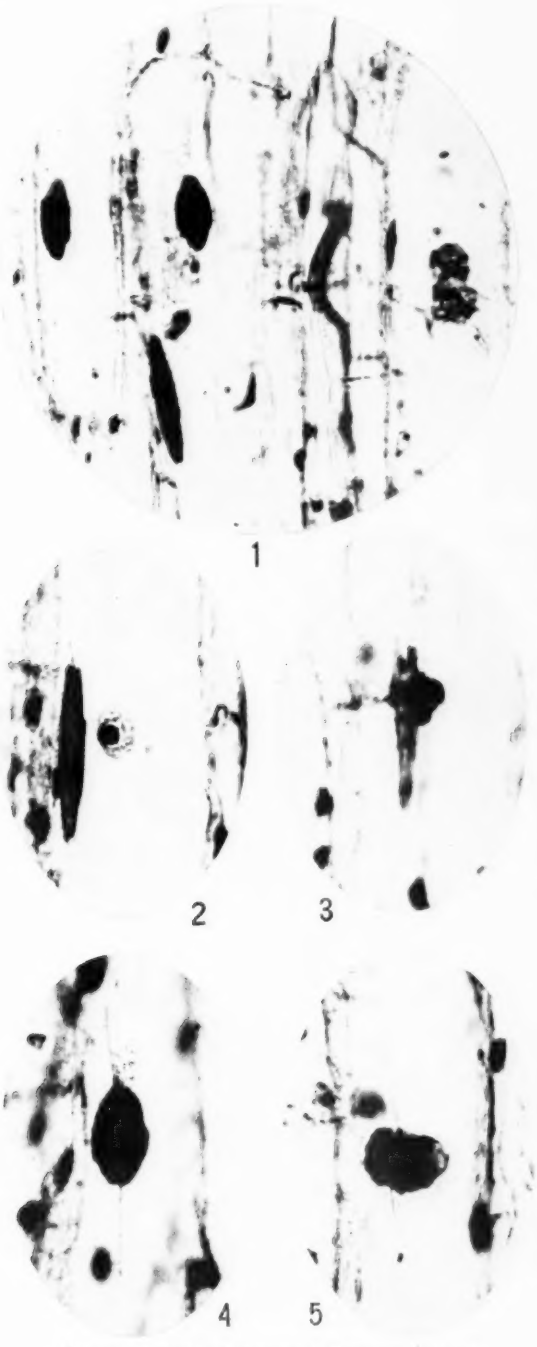
In connection with my work upon the cytology and histology of the lateral meristem, I have had occasion to collect carefully killed and fixed specimens of the cambium and phloem of the stem of *Robinia Pseudo-Acacia* L. As shown by Strasburger[1] (1891), this tree is characterized by having curious "slime-bodies" (Schleimklumpen) in its sieve tubes. The "slime-bodies," which stain intensively in Millon's reagent, vary greatly in size and shape during different stages in the differentiation of the sieve tubes. At an early stage, they are small, slender, spindle-shaped structures, but later they enlarge, assuming the various forms illustrated in plate XVI, figures 1–5. The larger, spindle-shaped masses, which frequently tend to be bifurcated at the ends (Pl. XVI, fig. 3), or more or less deeply cleft longitudinally, are held in place by slender threads attached at the ends of the spindle. As the "slime-bodies" enlarge laterally, these strands become thicker and more conspicuous (Pl. XVI, figs. 4 and 5).

That the organisms, described and figured by Nelson[2] in the phloem of bean and clover affected with mosaic are "slime-bodies," such as occur normally in the sieve tubes of Robinia and other Leguminosae, seems certain. Nelson emphasizes the extreme polymorphism in the life cycle of the supposed flagellates and cites the following principal forms:

(1) Large, elongated, biflagellate, tapering, or cigar-shaped individuals with the form of the body resembling organisms belonging to the genus Leptomonas, the flagella being attached at opposite poles. This is the most common type. (2) Ovaliform biflagellates of the same type. (3) Deep-staining, slightly elongated bodies, surrounded by a lighter envelope, with flagella running down to the centrally stained portion and projecting from each end of this destained envelope. Individuals of this type may be almost spherical and occur in pairs in the same cell (4) Very small, elongated flagellates, less than 0.5 micron in diameter and similar to type 1. These are common in sieve and parenchyma cells and probably represent very young forms of type 1. (5) Deeply stained oval bodies with degenerate flagella and occasional remnants of an outer envelope. These may be encysted forms.

[1] Strasburger, Edward. Histologische Beiträge, Heft **3**: 193–194. 1891.

[2] Nelson, Ray. The occurrence of protozoa in plants affected with mosaic and related diseases. Mich. Agr. Exp. Sta. Tech. Bull. **58**, p. 1–30, 18 figs., 1922.



SLIME-BODIES OF ROBINIA PSEUDO-ACACIA

The "slime-bodies" of Robinia Pseudo-Acacia take on all of these forms during various stages in the differentiation of the phloem. Types 1, 2, and 4 have already been referred to. The forms with a "destained" outer envelope occur commonly in nearly mature sieve tubes. The paired condition is illustrated in plate XVI, figure 1 (extreme right) Bifurcated or cleft "slime-bodies," suggesting stages in longitudinal fission, are of frequent occurrence. During the later stages in the enlargement and modification of the "slime-bodies," the sieve tube nuclei degenerate, as they do normally in most Gymnosperms and Angiosperms. The "slime-bodies" may, in certain cases, be in contact with the nuclei (Pl. XVI, fig. 3). Furthermore, although the smaller, spindle-shaped "slime-bodies" are relatively homogeneous, they may contain one or more deeply staining granules.

In discussing the identity of the structures described by Nelson, it should be emphasized that those figured by the writer are larger owing to the fact that they were obtained from stems of large trees. The cambial initials and their derivatives are larger in old robust stems than in young slender shoots, and the larger sieve tube elements tend to have larger "slime-bodies."

The movements recorded by Nelson in freshly cut sections of living material mounted in boiled water are to be expected. In removing the sections from the plant, the stresses and strains in the various tissues are changed, and the osmotic and other equilibria are disturbed upon emersion in water. That the "slime-bodies," attached as they are by long slender threads, should remain perfectly stationary during subsequent readjustments would be remarkable, even if the liquid or semi-liquid contents of the cells had no circulatory movements.

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DESCRIPTION OF PLATE XVI

Fig. 1. *Robinia Pseudo-Acacia* L. Tangential longitudinal section of phloem of stem of an old tree, showing spindle-shaped, curved and ovaliform "slime-bodies." × 416.

Fig. 2. *Robinia Pseudo-Acacia* L. Portion of sieve tube in tangential longitudinal section, showing "flagellate," spindle-shaped "slime-body" and nucleus with deeply stained nucleolus. × 690.

Fig. 3. *The Same.* Portion of sieve tube in tangential longitudinal section, showing spindle-shaped, bifurcated "slime-body" in contact with nucleus. × 690.

Fig. 4. *The Same.* Portion of sieve tube in tangential longitudinal section, showing "biflagellate," ovaliform "slime-body." × 690.

Fig. 5. *The Same.* Portion of sieve tube in tangential longitudinal section, showing "triflagellate," transversely expanded "slime-body." × 690.

FURTHER NOTES ON THE RELATION OF THE SPORE LOAD TO THE PER CENT OF STINKING SMUT APPEARING IN THE CROP

F. D. Heald and L. W. Boyle

In a previous contribution[1] the results were given of a study of the relation of spore-load, or the quantity of spores carried by each grain, to the percent of stinking smut appearing in the crop, for both naturally and artificially smutted seed. It was pointed out that the determination of the spore-load offered a method of predicting the percent of smut that would appear in the crop under optimum conditions provided no seed disinfection should be practiced, thus making possible a rational selection of wheat for seed purposes and also indicating whether seed treatment in any given case would be a paying procedure. Some additional results from experiments in progress at the time of writing the first article, are now available and will be presented, since it is not planned to carry out any further tests bearing on this problem.

In the spring of 1921 tests were made with the same two varieties, Marquis and Jenkins Club, as used in the previous experiments, with the addition of Early Baart, Hybrid 143, Little Club and Bluestem. One-hundred-gram lots of seed of each variety were smutted by shaking in a glass bottle with the respective amounts of smut, for lots carrying 0.04 to 3 grams. For lots of seed carrying 0.02 grams or less of smut, distribution of the spores was accomplished by making a suspension in water and spraying them over the grain with an atomizer. It was felt that the latter method insured a better distribution of the spores when such small quantities of smut were being used. The smut was distinctly evident by the coloration of the "brush" in all samples down to and including 0.08 grams of powdered smut to each 100 grams of seed, but was not visible except on white grains when less than 0.04 grams of smut was used. From the table 1 it may be noted that grain may be carrying several thousand smut spores per berry and still show no evidence of smut by naked eye inspection.

Two rod-rows of each lot of seed were planted by hand on the same day, using as nearly as possible uniform depth and spacing of seed, so that the different lots would be directly comparable. Plant and head counts were made at harvest time to determine the percent of smut in the different plots. Very soon after seeding time spore counts were

[1] Heald, F. D. The relation of spore load to the per cent of stinking smut appearing in the crop. Phytopathology **11**: 269–278. 1921.

made for each lot of seed using the Levy counting cell and mechanical stage and following the same method as in the previous work.[1]

The spore-load for the different varieties is given in the appended table (Table 1). The maximum number of spores per grain was 278,616 for

TABLE 1—*The number of spores per grain, using various weights of smut per 100 grams of seed.*

Weight of smut per 100 grams seed	Early Baart	Marquis	Jenkins Club	Hybrid 143	Little Club	Blusetem
3 grms.	263,973	263,116	152,441 (?)	163,675	237,619	278,616
2	181,480	210,626		120,016	164,798	209,872
1	84,033	100,402	75,795	61,297	81,578	102,440
0.8	63,689	76,864	73,671	50,710	68,224	77,698
0.4	27,539	39,491	33,404	28,912	31,716	40,331
0.25	17,909	19,677	18,678	17,659	24,673	23,337
0.1	6,926	6,843	6,510	5,429	12,521	7,925
0.08*	5,241	5,430	5,262	5,590	4,992	6,635
0.04**	1,976	1,997	2,371	1,716	1,664	3,161
0.02	1,019	1,061		499	1,144	1,289
0.01	499	561	1,227	644	686	624
0.005	312	478	936	457	416	416
0.00	104	21	790	228	229	0

Bluestem with three grams of smut per 100 grams of seed, while the lightest spore load varied from 312 to 936 per gram with 0.005 grams of smut per 100 grams of seed. The spore counts show a varying capacity of the different varieties for holding the smut powder, as may be noted by the smaller number in Jenkins Club and Hybrid 143. This variation may be due to the size of individual grains, the length and thickness of the brush, and other surface characters. It should be noted that all the unsmutted samples showed smut with the exception of Bluestem, which appeared to be smut-free.

The percent of smutted plants is given in table 2. With the exception of certain fluctuations which might be expected there was a gradual reduction in the percent of smutted plants with the diminished spore-loads. It may be noted that maximum smutting was obtained with one gram of smut per 100 grams of seed with Early Baart, Jenkins' Club and Little Club, while 2 grams of smut were required to produce maximum smutting with Marquis, Hybrid 143 and Bluestem. The portion of the table giving the results with spore-loads invisible or barely visible to the naked eye (.08 grams or less) is of special interest

[1] Loc. cit. p. 272-273.

* Smut distinctly visible to naked eye.

** Barely visible on white grains, but not on dark grains like Marquis.

as indicating the amount of smut which might be expected if visibly clean seed is selected for planting. With such samples the percent of

TABLE 2—*Per cent of smutted plants for different degrees of smutting.*

Weight of smut per 100 grams seed	Early Baart	Marquis	Jenkins Club	Hybrid 143	Little Club	Bluestem
3 grams	53.6	6.5	63.6	68.4	29.2	44.5
2	50.2	17.4	64.8	78.7	33.0	53.2
1	70.0	11.0	72.0	42.4	38.0	47.5
0.8	58.9	9.0	57.4	37.9	24.5	37.5
0.4	4.6	0.0	11.1	15.5	6.3	17.0
0.25	28.3	0.5	2.9	9.8	1.0	3.0
0.1	0.5	0.0	4.0	5.3	0.0	1.8
0.08	1.6	0.0	1.8	1.6	0.0	1.0
0.04	0.5	0.0	1.0	0.0	0.8	1.9
0.02	1.6	0.8	13.6	4.7	4.2	3.9
0.01	2.8	1.7	6.9	6.9	1.0	2.9
0.005	3.9	0.0	5.6	2.2	1.0	2.1
0.0	0.0	0.0	1.2	0.0	0.0	0.0

smut varied from 0 to 6.9 per cent with a single sample showing 13.6 per cent. Attention should be called to the pronounced jump in per cent of smut in all lots with spore-load of 0.02 grams per 100 grams of seed, and the gradual decrease again with the smaller spore-loads. This abrupt change can be explained only by the method of treating and distributing the spores. If all spores had been dusted on the grain there is every reason to believe that there would have been a gradual drop in the per cent of smut to the end of each series. In the previous paper it was shown that the per cent of smut appearing in the naturally smutted spring wheat is less than would be indicated by the spore-load as based on plantings of artifically smutted samples, this being due presumably to the fact that some of the seed-borne spores loose their power to germinate during the storage period of grain. The results shown in table 2 are then higher than would be expected under natural farm conditions, and suggest the question as to whether seed treatment of apparently clean seed is a paying proposition for spring plantings.

It was pointed out in the previous paper that seed treatment of such a resistant variety as Marquis is inadvisable when spring seeding is practiced, and it now seems probable that even susceptible varieties may be seeded in the spring without disinfection in case they are clean as far as naked eye examination can reveal, with an assurance that the per cent of smut in the crop will be very low.

This conclusion is borne out by the results shown in table 3. The only samples which carried a sufficient number of spores to be classed as "visibly smutty" were the two Early Baart lots with spore-loads of 6552 and 20,176. The per cents of smut in the "visibly clean" lots

TABLE 3—*Table showing the spore-load and per cent of smut from naturally smutted samples of wheat obtained from various farms. Spring, 1921.*

Variety	No. Samples	No. of Spores per grain	% Smutted plants
Bluestem	6	0, 21, 83, 104, 104, 145	0
"	1	104	0.85
"	1	145	0.9
"	1	166	0.5
"	1	375	0.5
Early Baart	5	0, 41, 42, 124, 229	0
"	1	966	1.0
"	1	6552	20.0
"	1	20,176	19.5
Marquis	6	0, 01, 21, 42, 104, 145	0
"	1	64	0.78
"	1	333	0.8
Hybrid 143	3	125, 125, 187	0
Jenkins Club	1	499	0
Red Chaff	1	270	0

ranged from 0 to 1 with spore-loads from 0 to 966. Since one per cent of smut would have little or no effect upon yield it is doubtful if seed treatment of any of the visibly clean seed would have been a paying proposition.

OVER-SUMMERING OF LEAF RUST OF CEREALS IN KENTUCKY

W. D. Valleau

The problem of determining the exact method of over-summering of the leaf and stem rusts of the small grains is an important one in Kentucky. It is comparatively easy to understand how the rusts over-winter once mycelium becomes established in the tissues of plants whose leaves live thru the winter. The small-grain fields go thru the winter often entirely free from either leaf or stem rust, but as conditions become favorable for infections in the spring, wheat fields especially become heavily infected first with the orange leaf rust and later with the black stem rust. This requires that the leaf rust at least must have over-summered the previous season in considerable abundance. The barberry has not been considered a factor in the black stem rust problem in Kentucky but as this disease is very prevalent late in the season on wheat it is possible that it may also over-summer in the same way as the orange leaf rust.

Volunteer wheat (or barley or rye) plants have been considered the host on which the leaf rusts over-winter but little detailed evidence has been published as to when the volunteer plants become established and how the rust is carried over from the infected crop to these plants. It is in an attempt to supply some of these details that this note is written.

In attempting to find suitable material for study of over-wintering of the black stem rust of wheat on the Kentucky Experiment Station farm during the fall of 1922, Mr. J. W. Barringer and the writer found a few plants of volunteer wheat infected with leaf rust in a clover field. As this suggested that volunteer wheat plants in clover fields might be the means of over-summering of both leaf and stem rusts, the writer made further studies of first year clover fields following barley, rye and wheat to determine more accurately the possible relationship between clover or grass fields sown in the small grains and the over-summering of the rusts.

Altho most of the observations have been made the past fall and winter, the history of the rusts from harvest time thru the following summer and winter seems to be so clearly written in the clover fields as to leave little doubt as to the exact method of over-summering.

The barley, rye and wheat in the fields studied was planted in the fall of 1921. In the early spring the fields were sowed to clover Some

time before harvest, the wheat and barley plants became heavily infected with orange leaf rust. Some lodging was observed during the spring in the barley and wheat fields. At harvest time, the lodged straw and some of the heads were crushed into the ground by the binder thus bringing seeds in close contact with the soil and leaving a mulch of straw over them. At the time the straw and leaves of all three grains bore numerous uredineal pustules of the orange leaf rust. Some of the wheat was also heavily infected with black stem rust. An inspection, in the fall, of the straw of some 50 varieties of wheat preserved in the seed house showed that the uredineal stage of the black stem rust was still present at harvest time. This condition seems usual in Kentucky as the writer has seen wheat fields at harvest time so heavily rusted that men and horses were covered with the brown spores of the black stem rust after running the binder for a short time. Mr. Barringer assured me that this is not the case further north in the winter wheat section. Some of each of the wheat, rye and barley seeds which had been crushed into the ground evidently germinated soon after harvest as evidenced by the fact that in the fall, numerous well established plants surrounded by small masses of dead rusted leaves were found under their respective mulches of straw. Other seeds germinated during the fall. Plants from these when examined late in the fall were characterized by having seedling leaves only and a poorly developed root system. These plants were rarely found to be rusted.

The young plants which developed in the early summer soon after harvest grew up thru the mulch of heavily rusted straw and evidently thus became infected. It is well known that the red rust spores of both the leaf rust and stem rust can survive for a considerable number of days if conditions are not right for germination and thus the primary infection of the volunteer plants may be accounted for. The writer was unable to find volunteer plants either of barley, wheat or rye which had lived thru the summer which were not fairly well covered with straw; while wherever the binder had crushed a mass of plants into the ground, volunteer plants which gave evidence of having over-summered could be readily found. They were always heavily infected with leaf rust. Following infection of the volunteer plants the process of over-summering of the orange leaf rust was very simple. The new leaves as they developed became infected from the older leaves which, in turn, died thus leaving the mass of dead leaves at the base of the plants in the fall. It is a comparatively simple thing the following spring for the leaf rust to spread from these heavily infected volunteer plants in the clover fields into neighboring wheat fields and to cause the severe epidemics which appear each year in this State.

Altho black stem rust of wheat has not yet been found over-summering in this manner by the writer, he sees no reason why it should not do so in view of the fact that uredinia are numerous at harvest time in some fields. If the fungus over-summers in this way, there is little question as to its ability to over-winter as mycelium in the leaves of these volunteer plants and thus account for the rather severe epidemics of black stem rust of wheat which often occur in Kentucky late in the spring.

An examination of rusted wheat, barley, timothy and orchard-grass during the winter has shown that most or possibly all of the leaves bearing rust pustules in the fall die during the winter; but apparently the rusts are capable of living through the winter as mycelium in vigorous green leaves as on March 4, 1923, the writer found a year old timothy plant partly protected by a brick and a light covering of straw which had several apparently newly formed pustules. These, when examined on March 14, appeared old, but a new fresh pustule containing viable spores was found on the same plant. On this date a volunteer barley plant which had lived through the previous summer was found on which there were 32 very small apparently new pustules of leaf rust on green, vigorous leaves. A single small pustule of orange leaf rust of wheat was found on a volunteer wheat plant March 8. All of the pustules found appeared to have resulted from mycelia which had over-wintered in green, vigorous leaves. They were all found in a one-year-old clover field.

As the practice of sowing grasses and clovers in wheat is general thruout much of the winter wheat area in this country, the clover and grass fields with their rusted volunteer wheat plants may be expected to play a prominent part in the over-wintering of the orange leaf rust and they may also prove to be a close rival of the barberry as a cause of epidemics of black stem rust in at least a portion of the winter wheat section.

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